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Original Article *Zoonoses*

Prevalence, virulence genes, and antibiotic resistance of Escherichia coli and Salmonella spp. isolated from pigeons and humans

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ABSTRACT

Objective: To investigate the prevalence of *Escherichia coli* and *Salmonella* spp. and their virulence genes and antimicrobial resistance in pigeons and humans in El-Dakahlia province, Egypt.
Design: Descriptive study.
Samples: A total of 274 samples were collected from pigeons (148 cloacal swabs, 20 feather swabs, and 26 egg samples), pigeons' environment (21 feed samples and 21 water samples), and their contact humans (20 hand swabs and 18 stool samples) in El- Dakahlia province, Egypt
Procedures: Cultural media, biochemical tests, and serotyping were used to isolate and identify *E. coli* and *Salmonella* from the collected samples. Moreover, multiplex-PCR for detecting virulence genes and antibiotic sensitivity tests were conducted on some *E. coli* and *Salmonella* isolates.
Results: The overall infection rates of *E. coli* and *Salmonella* spp. were 79.2% and 4.0%, respectively. Various serotypes of *E. coli* and *Salmonella* were recorded. Different virulence genes such as *stx1*, *stx2*, and *eaeA* were detected in 19 of 20 *E. coli* isolates, as well as *invA*, *hilA*, and *fimH* virulence genes were identified in 9 *Salmonella* isolates. The results of the antibiotic sensitivity test showed that all *E. coli* (n=20) and *Salmonella* (n=9) isolates were resistant to erythromycin, moreover, all nine *Salmonella* isolates were resistant to nalidixic acid.
Conclusion and clinical relevance: The results of this study indicate the role of pigeons in the transmission of both *E. coli* and *Salmonella* to humans. Therefore, strict hygienic measures should be taken into consideration when dealing with pigeons to diminish the potential transmission of both microorganisms.
Keywords: Pigeon, *E. coli*, *Salmonella*, Zoonoses, Human, Antibiogram profile.

1. INTRODUCTION

Pigeons (domestic and feral) are universally distributed in all urban and suburban areas of the world's entire countries, and their population growth is increased worldwide. Homing pigeons are pet birds that live in proximity and close contact with domestic animals and humans, facilitating and expediting the spread of zoonotic microorganisms [1]. Previously, the transmission of various pathogenic agents from diseased pigeons to different kinds of poultry has been explored [2]. Several and different zoonotic agents had been isolated and identified in pigeons [3]. Previous studies showed that feral and domestic pigeons were recognized as potential reservoirs of *Salmonella* spp. and *Escherichia coli* [4-6].

Multiplex PCR has been used to characterize enteric bacteria such as *E. coli* and *Salmonella* strains and their toxins and virulence genes using primers to identify more than one target sequence in a single reaction [7, 8]. Antimicrobial resistance (AMR) restrains the therapeutic potencies of treatment against bacterial infections in various species of domestic animals especially poultry [9]. Birds, including pigeons, may harbor strains of AMR microorganisms and distributed them, embracing risk to humans [10]**.** Several food-producing animals carry multidrug-resistant (MDR) strains of *E. coli* and *Salmonella* and become a protruding problem worldwide. It is potentially that animal hosts carrying antibiotic-resistant zoonotic microorganisms might enter through the food chain and environmental contamination to humans [11, 12]. Pigeons are deemed as one of the free-living birds, which may convey and carry many microbes with variable levels of antibiotic resistance [13, 14], leading to a rise in human pathogens resistance including, *E. coli* and *Salmonella* [15]. There are scarce peer-reviewed literature and publicly available data dealing with the role of pigeons and their environment as a considerable source and reservoir of zoonotic bacteria in Egypt. So, it was ultimately worth exploring the role of pigeons in harboring and disseminating the pathogenic *E. coli* and *Salmonella* strains that possess a significant public health hazard. Therefore, this study was designed to throw light on the isolation and identification of *E. coli* and *Salmonella* spp. from pigeons and their contact humans from El-Dakahlia province, Egypt using conventional and serological methods. Virulence genes of biochemically identified isolates were molecularly characterized by multiplex PCR, as well as their antimicrobial susceptibility tests were conducted.

2. MATERIALS AND METHODS

2.1. Ethics statement

The fieldwork associated with this study proceeded in acquiescence with the Guide for the Care and Use of Laboratory Animals in Egypt. The protocol of this study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt.

2.2. Sample collections

A total of 274 samples were randomly collected from pigeons (148 cloacal swabs, 20 feather swabs, and 26 egg samples either from fertile and non-fertile ones), pigeons' environment (21 feed samples and 21 water samples), and their contact humans (20 hand swabs and 18 stool samples) in El-Dakahlia Governorate, Egypt. All samples were obtained under sterile conditions then placed into a tube containing 0.1% buffered peptone water (BPW) (Oxoid, CM 1049), in an icebox, and then transferred to the laboratory of the Department of Hygiene and Zoonoses, Faculty of Veterinary Medicine, Mansoura University wherein, they were processed for isolation and identification of both *E. coli* and *Salmonella* species.

2.3. Isolation and Identification of E. coli and Salmonella spp.

E. coli and *Salmonella* spp. were isolated and identified as previously reported [16, 17]. In brief, a sterilized loop from the collected samples was streaked onto the surface of nutrient agar and incubated at 37 °C for 24 hours. Subcultures were then plated on MacConkey agar, eosin methylene blue (EMB) agar, xylose lysine deoxycholate (XLD) agar (Oxoid, CM0469B), and Salmonella-Shigella (SS) agar to obtain pure culture and cultural characteristics.

2.4. Biochemical test

Pure isolates were biochemically tested by using indole test, methyl red test, Voges–Proskauer (VP) test, citrate utilization test, urease test, triple sugar iron agar (TSI) test, Simmons' citrate agar test, and hydrogen peroxide 3% test [18].

2.5. Serological identification of E. coli and Salmonella:

The biochemically proved *E. coli* isolates were serologically identified as previously described [19], using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co., Japan). In addition, all *Salmonella* isolates were serotyped according to the Kaufmann-White scheme using O and H antisera (Difco) [20].

2.6. Molecular characterization of virulence genes of E. coli (n=20) and Salmonella (n=9) isolates

Twenty *E. coli* and nine *Salmonella* isolates were virtually examined by multiplex PCR for detection of virulence genes to zoonotic strains of *E. coli* (*stx1*, *stx2*, and *eaeA*), and *invA*, *hilA*, and *fimH* virulence genes in *Salmonella*.

2*.6.1. Genomic DNA extraction*

Extraction of genomic DNA from individual colonies were performed using geneJET genomic DNA purification kit (Fermentas) following the methods described [21]. The eluted DNA in 200 µL was stored at -20 °C until molecular analysis.

2.6.2. DNA Amplification for virulence genes of E. coli using multiplex PCR

Twenty confirmed *E. coli* isolates by biochemical and serological tests were subjected to PCR analysis for detection of a ~180-bp, 225-bp and 384-bp fragments of the *stx1*, *stx2* and *eaeA* genes, respectively as previously described [22]. PCR reactions were performed in a total volume of 25 µL containing 3 µM of oligonucleotides, 200 µM of each deoxynucleoside triphosphate, 3.5 mM MgCl2, 2.5 U of DNA Taq polymerase, and 1 µL of each primer (**Table 1**), 1 µL of Template DNA and then completed to 25 µL with nuclease-free water. The cycling protocol was started with initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 65 °C for the first 10 cycles, decrementing to 60°C by cycle 15 for 2 min and extension at 72 °C for 1.5 min., with a final extension at 72 °C for 10 minutes. Each PCR analysis was conducted in duplicate, using DNA of the *E. coli* isolated from the chicken as the positive control and reagent-grade water as the negative control. Five µL from each PCR amplified products were electrophoresed on 1.5% agarose gel stained with ethidium bromide solution, visualized, and photographed the visible bands using an ultraviolet transilluminator.

2.6.3. DNA amplification of virulence genes of Salmonella

The nine biochemically and serologically confirmed *Salmonella* isolates were screened by multiplex PCR protocol targeting ~284-bp, 497-bp and 1008-bp fragments of *invA, hilA* and *fimH* genes of *Salmonella*, respectively as previously described [23]. The PCR reactions were performed in 25 µL reaction volumes consisted of 2 µL of the bacterial DNA template, 10 µL of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl2, 2 µL of 10 mM dNTP mix 1.25 U of Taq DNA polymerase and 1 µL each of forward and reverse primer (10 pmol) (**table 1**) and completed to 25 µL with nuclease-free water. The PCR cycling protocol was performed as following: An initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 64°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. Each PCR analysis was conducted in duplicate, using DNA of the *S. enteritidis* isolated from the chicken as the positive control and reagent-grade water as the negative control. Finally, 5 µL of each amplicon was electrophoresed in 1.5 % agarose gel stained with ethidium bromide and visualized and captured on UV transilluminator.

2.7. Antibiotic sensitivity assay for E. coli (n=20) and Salmonella (n=9) isolates

The resistances of twenty *E. coli* and nine *Salmonella* isolates to amikacin (30µg), ampicillin (10µg), cefotaxime (30 µg), cephalothin (30 µg), cephradine (30 µg), ciprofloxacin (5 µg), clindamycin (10 µg), doxycycline (30 µg), erythromycin (15 µg), gentamycin (10 µg), nalidixic acid (30 µg), neomycin (20 µg), norocillin (25 µg), penicillin G (10 IU), tetracycline (30 µg), and sulphamethoxazole (1.25/23.75 µg) were determined by the disk diffusion method as previously illustrated in Clinical and Laboratory Standards Institute Guidelines [24].

2.8. Statistical analysis

The infection rate of *E. coli* and *Salmonella* spp. were assessed from the proportion of positive to the total number of examined samples, with the exact binomial confidence intervals of 95% (95 CI%).

3. RESULTS AND DISCUSSION

Pigeons may contribute as a candidate reservoir of zoonotic bacteria able to infect humans and other animals or/and birds, which constitute the human food chain [25]. *E. coli* and *Salmonella* are considered prevalent zoonotic diseases for humans. Pigeons play a role in transmitting *E. coli* and *Salmonella*, which creates considerable biological risk [26]. In the current study, **Table 2** showed that the overall prevalence of *E. coli* was 79.2% (217/274), of which 90.2% from pigeon samples, 42.9% from pigeons' environment, and 63.2% from humans in contact with pigeons. Moreover, the overall prevalence of *Salmonella* spp. was 4.0% (11 /274), of which 2.1% from pigeon samples, 4.8% pigeons' environment, and 13.1% from humans in contact with pigeons. No difference in the results was detected between the ratio of positive to the total number of examined samples. The prevalence of *E. coli* from pigeons in our study is greatly higher than those recorded in pigeons from Saudi Arabia (2.5%), India (4%), and Italy (7.8%) [26-28]. In Bangladesh, two studies found that the infection rate of *E. coli* in pigeons was 52.5% and 69.6% [5, 29]. The difference in prevalence may be attributed to the different diagnostic techniques used, different environments, climates, sample size, and regional variation [5]. The isolation rate of *Salmonella* from pigeons in the present study was slightly lower than what was detected in pigeons from Spain (4.4%) [30]. The prevalence of *Salmonella* from pigeon in earlier studies from Egypt were ranged from 4.8 to 13.3% [31, 32].The diversity in *Salmonella* isolation rates from pigeons might be related to the variances in serotypes and habitats [33]. Our results indicated that the isolation rate of *E. coli* from humans was higher than that recorded in humans from Thailand (53%), while in the same study, the isolation rate of *Salmonella* from humans was nearly similar (15%) [34]. Moreover, we found that the percent of *Salmonella* isolation from hand swabs (22.2%) was higher than that detected from stool samples (5.0%), this is in line with results of Ahmed et al. (2016) [35] who isolated a higher percent of *Salmonella* from hand swabs (10%) than that from stool samples (0.8%), so it can be recommended that cleaning and disinfection of hands before and after contact with pigeons are essential to reduce the likelihood of cross-contamination.

In our study, the most common serotype of twenty *E. coli* isolates was *E. coli* O78 isolated from two pigeon cloacal swabs, and one samples of each of egg samples, pigeon water samples, and human hand swabs then followed by *E. coli* O128:H2 that was isolated from pigeon cloacal samples and water samples, and human stool samples (one sample, each), as well as *E. coli* O91: H21 which was isolated from one sample of each of pigeon cloacal samples and feed sample, and human stool samples. Moreover, *E. coli* O26:H11 was isolated from human stool samples and hand swabs (**Table 3**). In addition, *E. coli* O2: H6 and O1: H7 were isolated from pigeon cloacal samples and pigeon egg samples, respectively. Nearly similar serotypes of *E. coli* were isolated from chickens, ducks, pigeons, and humans from El-Dakahlia province, Egypt [36]. Previous report showed that the avian pathogenic *E. coli* isolates especially, O1 and O2 have genetic commonalities and propinquities in virulence genes with neonatal meningitis *E. coli* and human uropathogenic *E. coli* and capabilities to cause meningitis and urinary tract infections in humans [37]. Meanwhile, the most common serotype of nine *Salmonella* isolates was *S. kentucky* (n=3) isolated from pigeon cloacal samples and egg samples, and human stool samples, followed by *S. enteritidis* (n=2) isolated from pigeon feed samples and human hand swabs, then one serotype for each *S. wingrove*, *S. typhimurium*, *S. larochelle*, and *S. tsevie* which were isolated from pigeon cloacal samples, pigeon water samples, pigeon water samples, and human hand swabs, respectively. In a previous study, *S. typhimurium*, *S. newport*, and *S. emek* were isolated from pigeons in Iraq [38]. In contrast to our results, the most common serotypes isolated from pigeons in Egypt was *S. typhimurium* [31, 32]. In another study from Egypt, *S. salamae* was the most common serotype of *Salmonella* isolated from pigeons [39]. Both *S. typhimurium and S. enteritidis* are responsible for the most human non-typhoidal salmonellosis [40]. Therefore, pigeons, particularly in high-density areas of pigeons may be a reservoir for zoonotic *E. coli* and *Salmonella*, and this may constitute a threat to human health.

Virulence genes are an array of factors that function synergistically to continue the growth of the microorganism within the host and abet it to show its virulence leading to the manifestation of the pathogenic process and the severity of the infection [41]. In this work, 20 *E. coli* isolates were subjected to multiplex PCR for detection of three virulence genes (**Figure 1**). The results indicated that stx*2* gene is the most prevalent gene (13/20, 65%), followed by the *stx1* gene (11/20, 55%), and the *eaeA* gene was detected in one isolate each among the 20 *E. coli* tested. In addition, three virulence genes were recorded in 9 *Salmonella* isolates. using multiplex PCR, the *invA* gene was detected in all 9 isolates (100%), while *hilA* and *fimH* genes were investigated in 7 (77.8%) and 6 (66.7%) of 9 isolates, respectively (**Figure 2**). In the same manner, previous study from Egypt detected *stx1*, *stx2*, and *eaeA* genes from *E. coli* isolates from chickens, ducks, pigeons, and humans [36]. The *invA* gene was widely used for the recognition of *Salmonella* spp. in different samples and was related to intestinal invasion [42]. In agreement with our results, *invA* gene was amplified in all examined *Salmonella* serovars isolated from chicken, pigeons, and humans in Egypt [35, 36]. These findings are worthy and perhaps a public health worry since these isolates have a pathogenic possibility to cause disease in humans [43].

The results of the antibiogram profile of 20 *E. coli* and 9 *Salmonella* spp isolates were presented in table (**4**). All the tested *E. coli* isolates (100%) were found to be resistant to erythromycin. Moreover, about 95%, 80%, 70%, 70%, 65%,

55%, 50%, 45%, 45%, 40%, 30%, 25%, 20%, 10%, and 5% of *E. coli* isolates were found to be resistant to cefotaxime, sulphamethoxazole, clindamycin, nalidixic acid, tetracycline, cephradine, penicillin G, norocillin, cephalothin, doxycycline, neomycin, amikacin, ampicillin, gentamycin, and ciprofloxacin, respectively. Similarly, all isolates of *Salmonella* spp. (100%) were found to be resistant to erythromycin and nalidixic acid. In addition, about 77.8%, 66.7%, 55.6%, 55.6%,

44.4%, 44.4%, 44.4%, 33.3%, 33.3%, 33.3%, 33.3%, 22.2%, 22.2%, and 11.1% of *Salmonella* isolates were found to be resistant to cephradine, norocillin, cefotaxime, sulphamethoxazole, clindamycin, penicillin G, cephalothin, tetracycline, neomycin, ampicillin, gentamicin, doxycycline, ciprofloxacin, and amikacin, respectively.

Table 1. Oligonucleotide primers' sequences used for identification of *E. coli* and *Salmonella*.

Target bacteria	Target primer	Primer Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
E. coli	*stx1*	(F) ATAAATCGCCATTCGTTGACTAC (R) AGAACGCCCACTGAGATCATC	180	[46]
	stx2	(F) **5′** GGCACTGTCTGAAACTGCTCC **′3** (R) **5′** TCGCCAGTTATCTGACATTCTG **′3**	255	
	eaeA	(F) GACCCGGCACAAGCATAAGC (R) CCACCTGCAGCAACAAGAGG	384	
Salmonella	*invA*	(F) GTGAAATTATCGCCACGTTCGGGCA (R) TCATCGCACCGTCAAAGGAACC	284	[47]
	hilA	(F) CTGCCGCAGTGTTAAGGATA (R) CTGTCGCCTTAATCGCATGT	497	[48]
	fimH	(F) GGA TCC ATG AAA ATA TAC TC (R) AAG CTT TTA ATC ATA ATC GAC TC	1008	[49]

Table 2. Incidence of *E. coli* and *Salmonella spp.* in pigeons, pigeons' environment, and in contact humans.

Samples		No. of sample collected	*E. coli*		*Salmonella* spp.	
			No. of positive (%)	95 CI%	No. of positive (%)	95 CI%
Pigeons	Cloacal swabs	148	144 (97.3)	0.94-0.99	3 (2.0)	-0.002- 0.042
	Feather swabs	20	16 (80.0)	0.62-0.97	0 (0.0)	0.00
	Egg samples	26	15 (57.7)	0.38-0.76	1 (3.8)	-0.035- 0.111
	Subtotal	**194**	**175 (90.2)**	**0.86-0.94**	**4 (2.1)**	**0.001- 0.041**
Pigeons' environment	Water samples	21	9 (42.9)	0.21-0.64	2 (9.5)	-0.030- 0.220
	Feed samples	21	9 (42.9)	0.21-0.64	0 (0.0)	0.00
	Subtotal	**42**	**18 (42.9)**	**0.27-0.57**	**2 (4.8)**	**-0.016- 0.112**
Humans	Stool samples	20	13 (65.0)	0.44-0.85	1 (5.0)	-0.045- 0.145
	Hand swabs	18	11 (61.1)	0.38-0.83	4 (22.2)	0.030- 0.413
	Subtotal	**38**	**24 (63.2)**	**0.47-0.78**	**5 (13.2)**	**0.023- 0.238**
Total		**274**	**217 (79.2)**		**11 (4.0)**	

The obtained results for both *E. coli* and *Salmonella* antibiogram profiles were nearly similar to that previously observed in Egypt [31, 36]. A previous study showed that all isolates of *E. coli* from pigeons in India (n=21) were sensitive to ciprofloxacin and 61.90% of *E. coli* isolates were resistant to erythromycin, in the same study, all *Salmonella* spp. (n=11) isolates were found to be resistant to tetracycline [5] .Our results differ from those obtained in Poland, where all *Salmonella* isolates were susceptible to enrofloxacin, amoxicillin with clavulanic acid, amoxicillin, flumequine, florfenicol, and trimethoprim/sulfamethoxazole [44]. The difference in antibiogram profiles between various studies may be attributed to the variance in the use of these antimicrobial drugs in different areas or due to the presence of distinct clones of *E. coli* and *Salmonella* in the study area [45]. Awareness should be directed to those antibiotics used in pigeons drinking water, pigeons feed, and as growth promoters in suboptimal doses. The observed high levels of resistance to antimicrobials reflected their common use in Egypt. It may be assumed that the great use of antimicrobials for the treatment and prevention of diseases without veterinary or medical consultation results in high levels of antibiotic resistance.

4. CONCLUSION

It could be concluded that pigeons from El- Dakahlia, Egypt are contaminated with *E. coli* and *Salmonella* spp., and many of these isolates bore zoonotic virulence genes, hence, pigeons and their environment constitute a significant problem for public health. Therefore, it is important to improve the management and hygienic practice measures, to decrease the hazard of infection with *E. coli* and *Salmonella* that could be spread from pigeons to humans.

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Conflict of interest statement

No conflict of interest.

Animal Ethics Committee permission

The current study is authorized to be carried out according to standards of the Research Ethics committee, Faculty of Veterinary Medicine, Mansoura University.

Authors' contributions

Ahmed El-Nagar carried out the lab work, Doaa Naguib conceptualized the study, planned for the research activity, analyzed data, and wrote the manuscript. Adel El-Gohary and Amro Mohamed planned for the research activity and revised the final version. All authors have read and approved the final version of the manuscript for publication.

Table 3. Serotypes of *E. coli* (n=20) and *Salmonella* spp. (n=9) from pigeons, pigeons' environment, and humans' samples.

Samples	*E. coli* serotypes (n)	*Salmonella* serotypes (n)
Pigeon cloacal samples	O2: H6 (1) O78 (2) O91: H21 (1) O128: H2 (1) O146: H21 (1) O159 (1)	*Salmonella wingrove* (1) *Salmonella kentucky* (1)
Pigeon egg samples	O1: H7 (1) O78 (1)	*Salmonella kentucky* (1)
Pigeon feed sample	O91: H21 (1)	*Salmonella enteritidis* (1)
Pigeon water samples	O78 (1) O128: H2 (1)	*Salmonella larochelle* (1) *Salmonella typhimurium* (1)
Human stool samples	O17: H18 (1) O26: H11(1) O55: H7 (1) O91: H21 (1) O128: H2 (1)	*Salmonella kentucky* (1)
Human hand swabs	O26: H11(1) O78 (1) O124 (1)	*Salmonella tsevie* (1) *Salmonella enteritidis* (1)

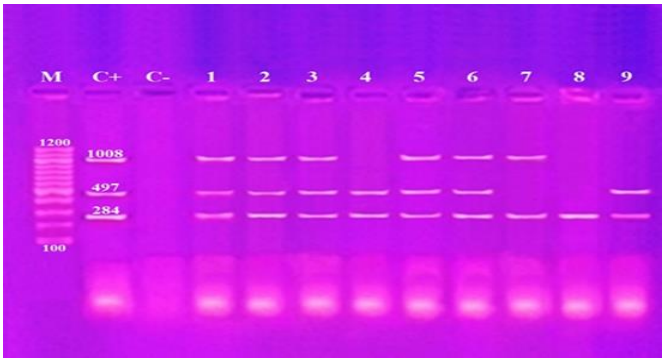

Figure 1. Agarose gel electrophoresis of multiplex PCR of *stx1* (180 bp), *stx2* (255 bp) and *eaeA* (384 bp) virulence genes for characterization of *Enteropathogenic E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for *stx1*, *stx2* and *eaeA* genes. Lane C-: Control negative. Lanes 1, 2, 3, 4, 5, 6, and 7 are pigeon cloacal swabs. Lanes 8 and 9 are pigeon egg samples. Lane 10 is pigeon feed sample. Lanes 11 and 12 are pigeon water samples. Lanes 13, 14, 15, 16, and 17 are human stool samples. Lanes 18, 19 and 20 are human hand swabs.

Table 4. Antibiogram profile of *E. coli* (n=20) and *Salmonella* spp. (n=9) to different antimicrobials.

Antimicrobial agent	Numbers of isolates showing resistant to antimicrobial	
	***E. coli* (%)**	***Salmonella* (%)**
Erythromycin (E)	20 (100)	9 (100)
Cefotaxime (CF)	19 (95)	5 (55.6)
Sulphamethoxazole (SXT)	16 (80)	5 (55.6)
Clindamycin (CL)	14 (70)	4 (44.4)
Nalidixic acid (NA)	14 (70)	9 (100)
Tetracycline (T)	13 (65)	3 (33.3)
Cephradine (CE)	11 (55)	7 (77.8)
Penicillin G (P)	10 (50)	4 (44.4)
Norocillin (NO)	9 (45)	6 (66.7)
Cephalothin (CN)	9 (45)	4 (44.4)
Doxycycline (DO)	8 (40)	2 (22.2)
Neomycin (N)	6 (30)	3 (33.3)
Amikacin (AK)	5 (25)	1 (11.1)
Ampicillin (AM)	4 (20)	3 (33.3)
Gentamycin (G)	2 (10)	3 (33.3)
Ciprofloxacin (CP)	1 (5)	2 (22.2)

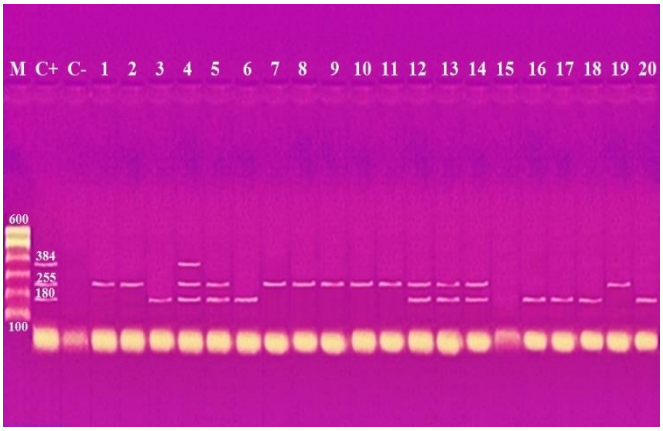

Figure 2. Agarose gel electrophoresis of multiplex PCR of *invA* (284 bp), *hilA* (497 bp) and *fimH* (1008 bp) virulence genes for characterization of *Salmonella* species. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive strain for *invA, hilA* and *fimH* genes. Lane C-: Control negative. Lanes 1 and 2 are pigeon cloacal samples. Lane 3 is pigeon egg sample. Lane 4 is pigeon feed sample. Lane 5 and 6 are pigeon water samples. Lane 7 is human stool sample. Lane 8 and 9 are human hand swabs.

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